

## Cloning of a Putative Ligand for the T1/ST2 Receptor\*

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**T1/ST2 is a receptor-like molecule homologous to the type I interleukin-1 receptor. Despite this sequence similarity, we have been unable to demonstrate binding of T1/ST2 to any of the three interleukin-1 species. In searching for a ligand for T1/ST2, we have cloned a cell surface protein to which it binds. This protein is unable to initiate signal transduction by the T1/ST2 receptor in several *in vitro* assays.**

The cytokine interleukin-1 exerts profound effects on a wide variety of cell types and tissues. These effects are mediated through a single receptor, the type I IL-1<sup>1</sup> receptor, which is comprised of an extracellular portion formed by three immunoglobulin domains, a single membrane-spanning segment, and a 215-amino acid cytoplasmic domain responsible for the signaling functions (1). In recent years, a number of homologs of the IL-1 receptor have been discovered. One of the most intriguing of these is a molecule called T1, ST2, or *fit-1* (referred to herein as T1/ST2), which has now been cloned independently three times on the basis of its strong induction by proliferative signals (2–4). These clones encode a secreted molecule possessing considerable sequence similarity to the extracellular, ligand-binding portion of both the type I and type II IL-1 receptors. Subsequently, a second form of T1/ST2 mRNA has been found, which encodes a transmembrane version of the same molecule, the cytoplasmic domain of which is also very similar to that of the type I IL-1 receptor (5). The T1/ST2 gene is tightly linked to the genes encoding the type I and type II IL-1 receptors in both mouse and human, and the intron/exon structures of murine T1/ST2 and human IL-1RI are virtually identical, providing further evidence that the two genes derive from a common ancestor (6, 7).

In adult mice and rats, the soluble and transmembrane versions of the T1/ST2 receptor are expressed predominantly in different tissues, a consequence of transcription from different promoters leading to the use of different polyadenylation sites and thereby to the production of different forms of mRNA (4, 8, 9). The soluble version is produced primarily by fibroblasts,

whereas the transmembrane version is found predominantly in hematopoietic tissues and in lung. During embryonic development, transmembrane receptor is again expressed predominantly in hematopoietic organs, whereas the soluble receptor is made in a limited set of tissues (skin, bone, eye) during a short period in the mid to late stages of gestation and then turned off again (8). Receptor protein is found in defined locations near to but not at the same place as receptor mRNA, suggesting that the soluble receptor diffuses away from the cells that make it until it encounters a ligand to which it can bind. Expression of soluble T1/ST2 receptor expression is also turned on in certain mammary tumor cells (4, 9, 10), consistent with its initial cloning as a gene induced upon stimulation of proliferation.

One attractive hypothesis, consistent with the above data, would postulate the existence of a ligand whose actions on the membrane-bound form of the T1/ST2 receptor are in some way incompatible with unrestrained cell proliferation. Soluble T1/ST2 receptor, induced upon stimulation of cell proliferation, could bind this ligand and prevent it from signaling through the membrane-bound receptor, thus freeing the way for cell growth.

We have attempted to identify a ligand for the T1/ST2 receptor. As part of this study, we have also asked whether T1/ST2 binds any of the IL-1 species, which seemed possible given the sequence similarity between T1/ST2 and IL-1RI. However, consistent with the absence in the literature of any evidence for the existence of IL-1 binding proteins other than the type I and type II receptors, we find no evidence for T1/ST2 binding to any of the IL-1 ligands. We have identified and cloned a protein that binds the T1/ST2 receptor but have not yet been able to demonstrate a functional response to this interaction.

### MATERIALS AND METHODS

Human and murine T1/ST2 extracellular regions were amplified by polymerase chain reaction from KG-1 and 3T3 cell RNA, respectively, and fused to human IgG1 sequences as described (11). The Ig portion was mutated to reduce binding to Fc receptors (12). The fusion protein in the vector pDC409 (13) was expressed in COS cells and purified on a protein A column.

A BIAcore biosensor (Pharmacia Biosensor) was used to examine binding of IL-1 ligands to the human T1/ST2 Fc fusion protein, essentially as described in detail in Arend *et al.* (14). Briefly, a goat anti-human IgG serum covalently coupled to the dextran matrix of a hydrogel chip was used to capture the human T1/ST2 Fc protein from concentrated COS cell supernatant. The appropriate IL-1 ligand, at 1  $\mu$ M concentration, was reacted with the captured protein, and the change of mass per unit area over time was measured.

Screening of cell lines for expression of T1/ST2 binding protein, determination of the affinity of binding of T1/ST2 Fc protein to A172 cells, and expression cloning of the binding protein from an A172 library were carried out as described (15, 16). The human binding protein cDNA was used as a probe to isolate murine binding protein clones from a 70Z/3 pre-B cell library by cross-hybridization. Two cDNA clones each were isolated for mouse and human binding protein; they differed in length by four (human) or five (mouse) nucleotides at the 5'-end, but those from a given species were otherwise identical throughout the coding region. The longer mouse clone appears to be missing three

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U41804, U41805.

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<sup>1</sup> The abbreviations used are: IL-1, interleukin 1; IL-1RI, type I interleukin 1 receptor.

N-terminal amino acids by comparison with the human clones.

For chromosome mapping, metaphase spreads from normal human lymphocytes were prepared according to the method of Fan *et al.* (17). The human T1/ST2 binding protein cDNA was labeled with biotin-16-dUTP (Boehringer Mannheim) by nick translation. Fluorescence *in situ* hybridization and detection of immunofluorescence were performed according to the technique of Pinkel *et al.* (18) with minor modifications (19). The chromosome preparations were stained with both diamidino-2-phenylindole and propidium iodide (Oncor) (17) and observed with a Zeiss Axiophot fluorescence microscope. Hybridization of the 1.3-kilobase cDNA probe to human chromosomes was performed on three separate occasions and revealed specific labeling on chromosome 19. Fluorescent signals were detected on chromosome 19 in 62 of 82 metaphase spreads examined. Nonspecific hybridization was moderately low, and 153 of 284 signals (53.9%) were located on chromosome 19. All signals on chromosome 19 were located at 19p13, with most appearing to reside at or beside sub-band 19p13.2.

To analyze expression patterns, RNA samples (5 µg) were electrophoresed, transferred to nylon membranes, and hybridized as described (16) to antisense riboprobes made from human (Fig. 4A) or mouse (Fig. 4B) T1/ST2 binding protein cDNA. Evenness of loading was monitored

by staining the filters with methylene blue following transfer. In Fig. 4C, a Clontech blot (catalogue no. 7760-1) containing 2 µg of poly(A)<sup>+</sup> RNA in each lane was hybridized to the human riboprobe.

Soluble human T1/ST2 binding protein expression constructs were made in pDC409 by fusing the first 194 amino acids (Met...Glu-Arg-Val-Asn) at their C terminus to either the human IgG1 Fc mutein (see above) or to the epitope tag FLAG (20). The equivalent murine versions were made by including the first three amino acids of the human binding protein to substitute for the missing murine N terminus. Proteins were expressed in CV1/EBNA cells (16) and either used directly as cell supernatant, with or without concentration, or purified on a protein G column (Fc construct) or an M2 anti-FLAG antibody column (FLAG construct) followed by elution with 12.5 mM sodium citrate, pH 2.8, and subsequent neutralization.

Construction of the reporter plasmid containing nucleotides -130 to +44 of the human IL-8 promoter (21) fused to the IL-2Rα chain coding sequence, transfection and stimulation of COS cells, and measurement of IL-2Rα chain expression by use of the 2A3 anti-IL2Rα antibody (22) followed by <sup>125</sup>I-goat anti-mouse Ig serum will be described in detail elsewhere.<sup>2</sup> The basic cassette (MuIL1R/-), containing the murine IL-1RI extracellular and transmembrane regions with no cytoplasmic portion, and the MuIL1R/ST2 chimera (mouse IL-1RI extracellular and transmembrane regions fused to the mouse T1/ST2 cytoplasmic domain) are described in Mitcham *et al.* (23). For the chimera containing the murine T1/ST2 extracellular portion, amino acids 1-358 of the full-length murine ST2 protein (5) were fused to amino acids 363-576 of the mouse type I IL-1 receptor (numbering is from the initiating methionine). The equivalent human chimera contained amino acids 1-334 of the human T1/ST2 protein, the transmembrane region (amino acids 330-358) of the full-length murine ST2 protein (since no sequence is available for the transmembrane version of the human receptor), and the amino acids 363-576 (the cytoplasmic portion) of the mouse type I IL-1 receptor. All chimeras were constructed in the expression plasmid pDC304, a variant of pDC302 (24). Stimulations of transfected COS cells were performed with 1 ng/ml of human IL-1α or with either 50 ng/ml or 1 µg/ml of human T1/ST2 binding protein Fc with equivalent results. Similarly, the presence or absence of the sheep anti-human IL-1RI antiserum P3 had no effect on stimulations using human T1/ST2 binding protein Fc.

## RESULTS

**T1/ST2 Receptor Does Not Bind IL-1**—To ask whether the T1/ST2 receptor bound to any of the IL-1 ligands, we fused the postulated extracellular portion of human T1/ST2 to the Fc segment of human IgG1 and expressed the resulting hybrid molecule in monkey kidney cells (see "Materials and Methods"). The Fc fusion protein was purified from the supernatant using protein A-Sepharose. Human T1/ST2 Fc fusion protein was immobilized on a BIAcore chip and exposed in turn to each of

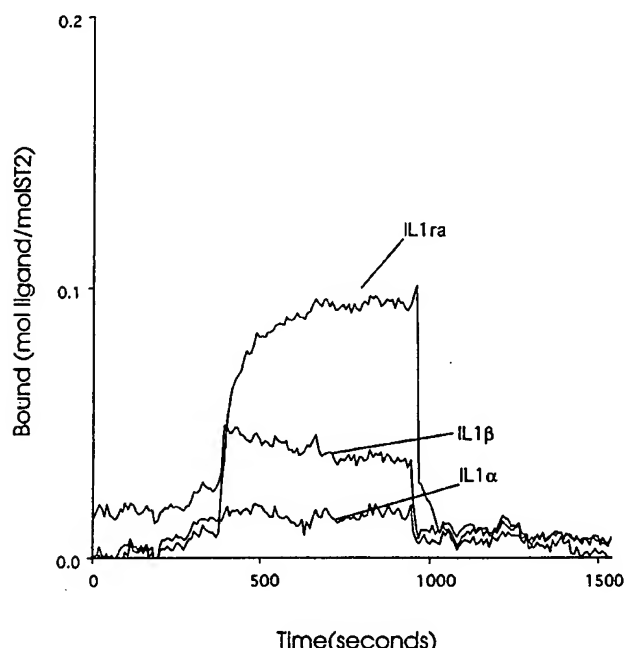


FIG. 1. Lack of binding of IL-1α, IL-1β, and IL-1ra to the T1/ST2 receptor, measured by BIAcore.

<sup>2</sup> T. P. Bonnert and J. E. Sims, manuscript in preparation.

TABLE I

Expression of T1/ST2 binding protein measured by flow cytometry on cells stained with T1/ST2 receptor Fc

Summary of flow cytometry data evaluating expression of T1/ST2 binding protein on various cell lines. Cells were stained with human T1/ST2 binding protein Fc fusion, followed by incubation with biotinylated mouse anti-human IgG and streptavidin-phycoerythrin. Included in the incubations as blocking reagents were 10% fetal bovine serum, 10% normal goat serum, 10% normal rabbit serum, and 50 µg/ml murine IgG. Human IL-4 receptor Fc fusion protein was used as the negative control. FACS, fluorescence-activated cell sorter.

FACS positive cells	FACS negative cells
Human neuroblastoma (A172)	Human T cell line (Jurkat)
Human astrocytoma (CCF-STTG1)	Human T cell clones (PL-1, PL-2, Cl22)
Human glioma (HS683)	Human T cell lymphomas (DG7S, Mac2a, HSC-M1, IMHOC7, DOHH-2, DG38, FL-LCAL, L548)
Human follicular dendritic cell (FDC2)	Human B cell line (Daudi, MP-1)
Human B cell lines (Raji, Namalwa)	Human monocytic cell line (THP-1)
Human myeloma (U266)	Human umbilical vein endothelial cells
Human lung fibroblasts (MRC5, MRC9, WI26VA4)	Human NK-like line (YT)
Human hepatomas (HepG2, SKHep)	Murine T cell line (5F4, CD6, D10, EL4, QH5)
Human fibrosarcoma (HS913T)	Murine myeloid line (M1)
Human lung adenocarcinoma (Calu 6)	Murine fetal liver Thy1 <sup>+</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> cells
Murine pre-B cell line (70Z/3)	Rat neural cell line (R1, R6)
Murine T lymphoma (Ox49.4, variant of S49)	
Murine gut cell lines (13-1, 13-3, 13-10, 2-6)	
Rat neural cell lines (B28, B133, B201)	
Rat glial tumors (B156, B200)	
Rat optic nerve tumor (R5)	

the three human IL-1 ligands (IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1 $\gamma$ ).

The only measurable binding in the experiment was observed with IL-1 $\gamma$  as a ligand (Fig. 1) and represented less than 10% occupancy of the T1/ST2 Fc fusion protein at 1  $\mu$ M ligand. The apparent rate of dissociation was too fast to measure. These data correspond to a  $K_d$  in the range of  $10^4$ – $10^5$  M $^{-1}$ , which is too low to be physiologically significant and which may simply represent nonspecific interaction of the ligand with the chip surface, with the goat anti-human IgG used for immobilization, or with the Fc portion of the fusion protein. With IL-1 $\alpha$  and IL-1 $\beta$ , the BIAcore traces showed no evidence of binding whatsoever. We conclude that the T1/ST2 receptor does not bind in a significant way to any of the known IL-1 family members.

**Cloning of a Ligand for T1/ST2**—To search for a potential T1/ST2 ligand, we screened a number of cell lines by flow cytometry for their ability to bind the human receptor Fc fusion protein. As negative controls, we used either the murine IL-4 receptor or the rat T cell antigen OX40 fused to the same Fc segment. Several cell lines were found to bind specifically to the T1/ST2 receptor (Table I). We characterized in more detail the binding to one of them, the human glioblastoma A172 (Fig. 2A). The T1/ST2 Fc fusion protein demonstrated saturable binding and gave a biphasic Scatchard plot (Fig. 2B). There were 1945 high affinity sites with a  $K_d$  of  $>1 \times 10^{11}$  M $^{-1}$  and 26358 intermediate affinity sites exhibiting a  $K_d$  of  $2.6 \times 10^8$  M $^{-1}$ . The A172 cell line thus seemed a good source from which to clone the putative T1/ST2 ligand.

To isolate cDNA clones of the T1/ST2 binding protein, we used an expression cloning strategy described previously (15, 16). A cDNA library from the A172 cell line was generated in the mammalian expression vector pDC410 (25). The library was transfected in pools of 2000 into CV1/EBNA cells growing on slides, and after 2 days the cells were incubated with the T1/ST2 receptor Fc fusion protein followed by a second step of  $^{125}$ I-rabbit anti-human IgG. The slides were coated with photographic emulsion, exposed, and screened under a low-power microscope for the presence of silver grains superimposed on cells, indicating expression of a cDNA capable of binding to the T1/ST2 Fc fusion protein.

Two positive clones were found after screening  $5 \times 10^5$  clones of an oligo(dT) primed library. The cDNA sequence predicts a type I transmembrane protein of 227 amino acids, with a 170-amino acid extracellular portion lying between a potential signal peptide and the transmembrane domain (Fig. 3). The protein also contains a 12-amino acid cytoplasmic tail. No similarity to any of the IL-1 ligands is apparent from the sequence of this clone nor is there any resemblance between the hydrophobicity plots, as is found for example between IL-1 $\alpha$  and - $\beta$  and acidic fibroblast growth factor (26). However, there are a number of open reading frames as well as expressed sequence tags in the DNA sequence data bases that show significant homology to the T1/ST2 binding protein (see legend to Fig. 3). One of these has been established to be an endosomal protein in yeast and another a microsomal protein in dog pancreas; the others are uncharacterized. Northern blots reveal that the  $\approx 1.5$ -kilobase T1/ST2 binding protein mRNA is expressed widely (Fig. 4). The gene encoding the human T1/ST2 binding protein was mapped to chromosome 19p13.2 (data not shown; see "Materials and Methods").

The human T1/ST2 binding protein cDNA clone was used as a probe to isolate a murine counterpart from the 70Z/3 pre-B cell line. The mouse sequence is very similar to the human one, showing 95% amino acid identity overall and 97% identity within the extracellular portion of the protein. Both human and mouse T1/ST2 receptor Fc fusion proteins bound to both human

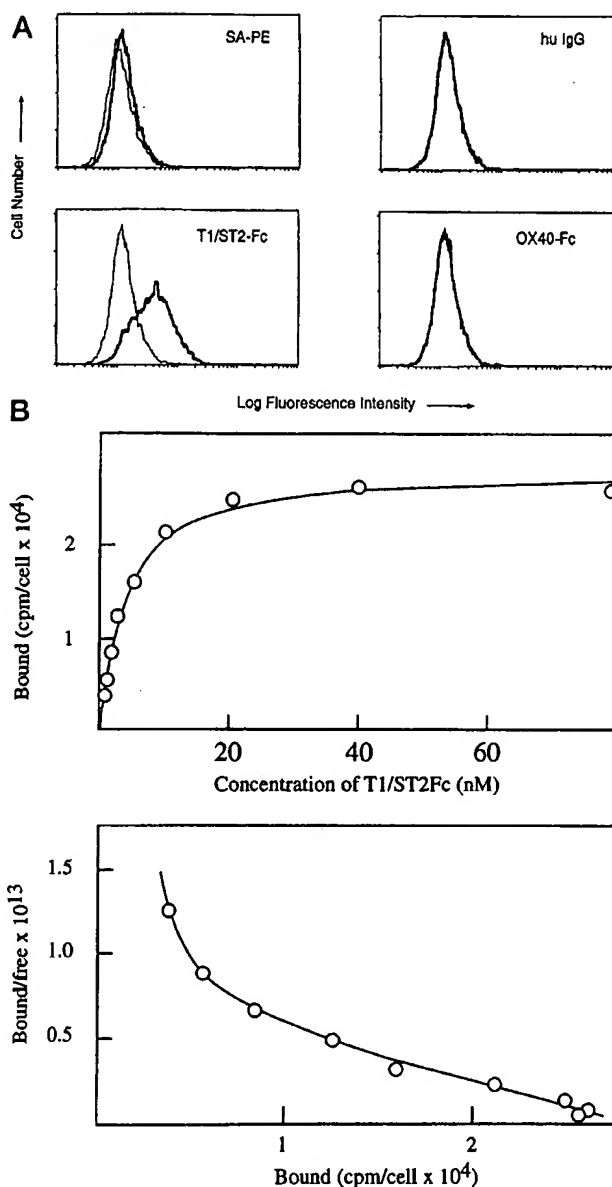


Fig. 2. A, flow cytometry profiles showing that human T1/ST2 Fc protein binds specifically to A172 cells. SA-PE, streptavidin-phycoerythrin detection reagent. B, binding curve (top) and Scatchard plot (bottom) of human T1/ST2 Fc protein binding to A172 cells. Cells were incubated with various concentrations of T1/ST2 Fc protein, and binding was subsequently detected using a saturating concentration of  $^{125}$ I-mouse anti-human IgG (15). The deduced site numbers and affinities are  $R_1$ , 1945 sites/cell;  $K_{d1}$ ,  $>1 \times 10^{11}$  M $^{-1}$ ;  $R_2$ , 26358 sites/cell;  $K_{d2}$ ,  $2.6 \times 10^8$  M $^{-1}$ .

and mouse binding proteins when the latter were expressed transiently in COS cells (data not shown).

To facilitate further studies, tagged versions of the binding proteins were made by fusing either the human IgG1 Fc moiety, described above, or the FLAG sequence (DYKDDDDK) (20) to the C terminus of their extracellular portions. It seemed likely that these tags would not interfere with function, since normally the extracellular portion is joined to its own transmembrane region at the same point. Both varieties of fusion construct, for both human and mouse binding proteins, showed good binding to mouse 3T3 cells in fluorescence-activated cell sorter experiments (Fig. 5; data not shown).

**Functional Assays**—We sought to demonstrate a function for

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human 1  MHAAGAAALALALMLLPFFVEVGAGPPFIQDGEFTLLPAGRKQCFYQSAFANASLETETQVIGGAGLDVFTLES PQGVLLVSESRKADGVRTVEPTAGDYKLCFDSFSTIS
mouse 1  ...AGAVLALALMLLPVGVGAGPPFIQDGEFTLLPAGRKQCFYQSAFANASLETETQVIGGAGLDVFTLES PQGVLLVSESRKADGVRTVEPTAGDYKLCFDSFSTIS

human 116 EKLVFFELIFDSIQDDEVEGNAAVEPECHLDVQEDIKESISTHRTKLSIQMLTLRAFEARDNLQGNLERNVNSAVVAVLLVAVLQVCTLGRFFQDKAPVPT
mouse 113 EKLVFFELIFDSIQDDEVEGNAAVEPECHLDVQEDIKESISTHRTKLSIQMLTLRAFEARDNLQGNLERNVNSAAVAVLLVAVLQVCTLGRFFQDKAPVPT

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FIG. 3. The amino acid sequences of the human (top) and murine (bottom) T1/ST2 binding proteins, as deduced from the cDNA clones. Identities are indicated by vertical lines. The murine clones appear to be missing the N-terminal portion of the signal sequence. The predicted site of signal peptide cleavage is indicated by an arrow head. The predicted transmembrane segment is underlined. There are a number of homologous cDNA sequences in the NCBI data bases. Four of these are human expressed sequence tags (three of them represented multiple times), which can be retrieved using accession numbers T17481, T48838, and T48839; T27390 and F06012; R54717, H03613, H27167, and H27168; and R54718. The others are a dog microsomal membrane protein (X53592) (29), a Chinese hamster coated vesicle protein (U26264), which is a homolog of the second group of human expressed sequence tags, a yeast endosomal protein (X67317) (30), and four open reading frames found by yeast genomic sequencing of chromosome I (L22015, translation of complement 4145–4792), chromosome IV (Z48432, translation of complement 18667–19344), chromosome VIII (U00059, translation of 18834–19472), and chromosome XV (X87331, translation of complement 9721–10344). An open reading frame on yeast chromosome XIII (Z49810, translation of 10178–10813) may also be a family member.

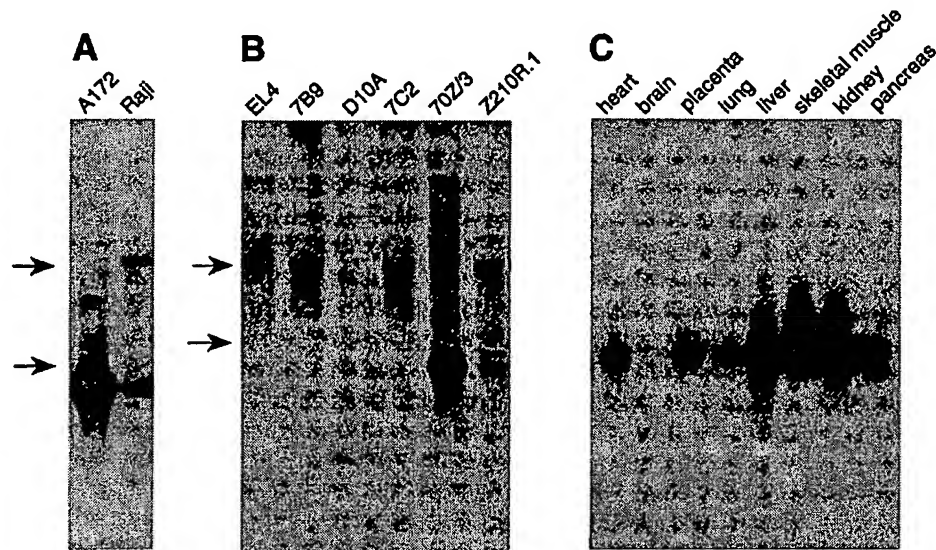


FIG. 4. Northern blots using antisense riboprobes from human (A and C) or mouse (B) T1/ST2 binding protein cDNA clones on the corresponding RNA. In A and B, poly(A)<sup>+</sup> RNA was used for the A172, EL4, and 70Z/3 samples; the other lanes contained total RNA. The arrows represent the positions of 28S and 18S rRNA. All RNAs in C are poly(A)<sup>+</sup>.

the T1/ST2 binding protein in two separate assays, both of which have been demonstrated to be responsive to signaling by the T1/ST2 cytoplasmic domain. The first assay is induction of DNA binding activity by the transcription factor NF $\kappa$ B in murine 3T3 cells, which have been reported to express mRNA for full-length (membrane-bound) T1/ST2 receptor (5). We have confirmed receptor expression in 3T3 cells by Northern blot and by reverse transcriptase-polymerase chain reaction using primers specific for the cytoplasmic domain of the receptor (data not shown) as well as by cell surface staining with the binding protein Fc fusion (Fig. 5). In experiments to be published elsewhere, we have shown that the T1/ST2 cytoplasmic domain, when fused to the murine IL-1 receptor extracellular and transmembrane regions and transfected into COS cells, is capable of activating NF $\kappa$ B in response to IL-1 treatment (23). It therefore seems reasonable to assume that a true ligand for T1/ST2, acting on the natural full-length receptor, will be capable of activating NF $\kappa$ B. Nevertheless, the T1/ST2 binding protein failed to activate NF $\kappa$ B in 3T3 cells, regardless of whether the stimulation was provided by the (dimeric) purified Fc fusion (Fig. 6) or the monomeric, unpurified FLAG version in COS cell supernatants (data not shown). Both of these reagents were shown by fluorescence-activated cell sorter analysis to be capable of binding to the 3T3 cells (Fig. 5 and data not shown). In addition, 70Z/3 cells, which naturally express T1/ST2 binding protein as judged by cell surface staining with T1/ST2 receptor Fc and from which the murine binding protein was cloned,

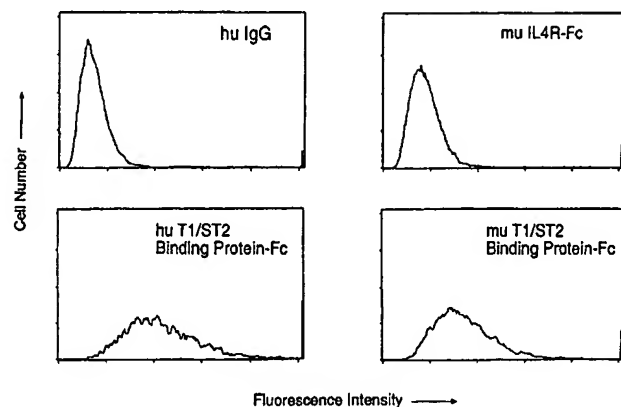


FIG. 5. Flow cytometry profiles of murine 3T3 fibroblasts following staining with human or mouse T1/ST2 binding protein Fc. Human IgG and murine IL-4 receptor Fc protein were used as negative controls.

failed to induce activation of NF $\kappa$ B when incubated with 3T3 cells (data not shown). NF $\kappa$ B DNA binding activity was induced in 3T3 cells by IL-1 (Fig. 6), demonstrating that the signaling pathway is intact in these cells.

A second assay used to assess functional activity of the T1/ST2 binding protein is the ability to stimulate transcription from the IL-8 promoter. In this assay,<sup>2</sup> the human IL-8 pro-



FIG. 6. Failure to activate DNA binding by NF $\kappa$ B upon stimulation of 3T3 cells with T1/ST2 binding protein Fc fusion. 3T3 cells were stimulated with the following: the negative control, murine IL-4 receptor Fc (lane 1, 250 ng/ml; lane 2, 10 ng/ml); the positive control, human IL-1 $\beta$  (lanes 3, 6, 7, 10 ng/ml; lanes 8, 9, 250 ng/ml); human T1/ST2 binding protein Fc (lane 10, 10 ng/ml; lane 11, 250 ng/ml); or murine T1/ST2 binding protein Fc (lane 12, 10 ng/ml; lane 13, 250 ng/ml). No extract was added to the probe in lane 5. In lanes 7 and 9, a 100-fold excess of unlabeled NF $\kappa$ B oligonucleotide was added as competitor to demonstrate specificity. NF $\kappa$ B electrophoretic mobility shift assays were conducted as described (31).

motor has been fused to the coding region of the human IL-2 receptor  $\alpha$  chain. The reporter construct is transfected into COS cells along with an appropriate receptor chimera, the cells are stimulated with ligand, and expression of IL-2R $\alpha$  on the cell surface is measured using  $^{125}$ I-labeled anti-IL-2R $\alpha$  antibody. Transcription of the transfected IL-8 promoter/IL-2R $\alpha$  chain reporter can be stimulated by IL-1 through either the endogenous COS cell IL-1 receptors (not shown) or, as can be seen in Fig. 7, through a transfected murine IL-1 receptor after antibody blocking of the endogenous receptors. Moreover, reporter expression can be stimulated by IL-1 through a transfected chimeric receptor containing the extracellular and transmembrane segments of the murine IL-1 receptor fused to the cytoplasmic portion of the murine T1/ST2 receptor (Fig. 7; Ref. 23). However, neither IL-1 nor the T1/ST2 binding protein was able to induce transcription of the reporter construct through the reverse chimera containing the extracellular and transmembrane segments of the murine T1/ST2 receptor fused to the cytoplasmic portion of the murine IL-1 receptor. Human IL-1 $\alpha$  also failed to stimulate the IL-8 promoter when tested on a comparable chimera containing the extracellular portion of human T1/ST2, confirming once again that IL-1 $\alpha$  is not a ligand for T1/ST2. Cell surface expression of the chimeric receptors was confirmed by flow cytometry after staining with either antibody or T1/ST2 binding protein Fc (data not shown).

#### DISCUSSION

Despite its considerable homology to type I and type II IL-1 receptors, we find that the T1/ST2 protein does not have any measurable affinity for any of the three known IL-1 species (IL-1 $\alpha$ , IL-1 $\beta$ , IL-1 $\gamma$ ). Other researchers have also failed to find binding to IL-1 $\alpha$  or IL-1 $\beta$  (8). One group (27) has reported a low binding affinity for IL-1 $\beta$ ; we have not been able to confirm this. Recently another IL-1 receptor homolog, called IL-1 receptor accessory protein (AcP), has been reported (28). The AcP associates with the type I IL-1 receptor and increases its affinity for IL-1; it may also play a role in IL-1 signal transduction. It is conceivable that the T1/ST2 protein functions similarly, although its restricted tissue distribution would suggest that it cannot be required for IL-1 signaling.

We have attempted to test another hypothesis, namely, that the T1/ST2 receptor has a ligand(s) of its own, capable of

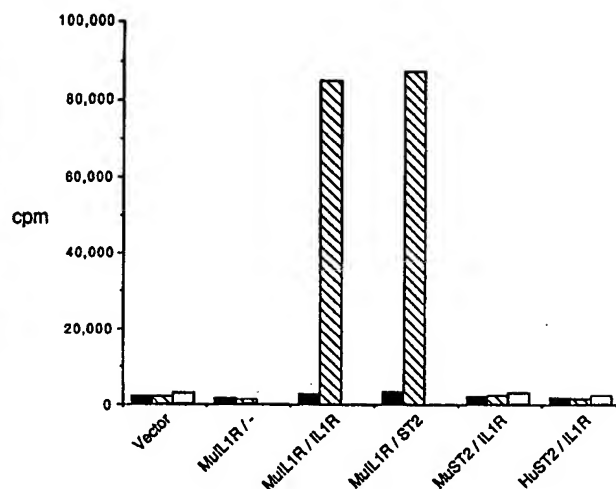


FIG. 7. Failure of T1/ST2 binding protein to activate transcription from the IL-8 promoter. COS cells were transfected with the indicated receptor plasmids together with a reporter plasmid containing the IL-8 promoter driving expression of the IL-2 receptor  $\alpha$  chain cDNA. 24 h later, the cells were stimulated overnight with medium (solid bars), 1 ng/ml IL-1 $\alpha$  (hatched bars), or 1  $\mu$ g/ml human T1/ST2 binding protein Fc (stippled bars). They were then incubated with mouse monoclonal antibody 2A3 against IL-2R $\alpha$  followed by  $^{125}$ I-goat anti-mouse Ig serum and counted. Stimulation was done in the presence of a 1/100 dilution of sheep anti-human IL-1RI serum P3 (23), which at this concentration blocks binding of IL-1 to the endogenous COS cell IL-1 receptors but has no effect on binding to the transfected mouse IL-1RI extracellular region. The P3 serum does not bind to human or mouse T1/ST2 (data not shown). The designations (X/Y) of the transfected receptor plasmids refer to the origin of the extracellular and transmembrane regions (X) and cytoplasmic domain (Y) of the chimera (see "Materials and Methods"); for example, MuL1R/ST2 contains the mouse IL-1RI extracellular and transmembrane regions fused to the cytoplasmic portion of mouse T1/ST2. In other experiments, human and mouse IL-1RI cytoplasmic domains were found to function equally well in this assay.

eliciting biological responses in receptor-bearing cells. Making use of a receptor/Fc fusion protein, we have cloned a cell surface molecule capable of binding to T1/ST2. The binding protein shows no sequence similarity to any of the IL-1 species. It does appear to be widely expressed and to possess homology to a number of other mostly uncharacterized open reading frames or cDNAs. We have tested the binding protein for biological activity in two assays, activation of DNA binding by NF $\kappa$ B and induction of transcription from the IL-8 promoter. Both of these assays respond well to IL-1, acting either through the type I IL-1 receptor or through a chimeric receptor consisting of the extracellular and transmembrane portions of the IL-1R fused to the cytoplasmic domain of T1/ST2. Neither responded to the T1/ST2 binding protein. In the NF $\kappa$ B assay, the endogenously expressed T1/ST2 receptor was used, whereas the IL-8 assay used a chimeric receptor in which the T1/ST2 extracellular portion was fused to the IL-1R cytoplasmic part. We have also asked whether the T1/ST2 binding protein has any activity in a number of assays of immune and hematopoietic function with consistently negative results (data not shown).

The assays used to measure biological function were chosen because we had good reason to believe that a ligand for T1/ST2 should read out in them. The T1/ST2 cytoplasmic domain has been demonstrated to be capable of leading to NF $\kappa$ B activation in the context of a chimeric receptor containing the IL-1R extracellular portion. It is reasonable then to assume that natural T1/ST2 receptor, responding to its true ligand, would also lead to NF $\kappa$ B activation. 3T3 cells, in which this assay was done, express natural T1/ST2 receptors and are capable of activating NF $\kappa$ B in response to other stimuli. Similarly, in the

COS cell assay, the IL-1R cytoplasmic domain is capable of activating the IL-8 promoter in the context of the endogenous monkey IL-1RI, a transfected mouse IL-1RI, or a chimeric receptor fusing the mouse IL-1RI extracellular and transmembrane portions to the human IL-1RI cytoplasmic domain. It is reasonable to assume that it will also be capable of eliciting IL-8 promoter activity when present in a properly activated chimera, which fuses it to the T1/ST2 extracellular and transmembrane portions, especially since the reverse chimera (IL-1R extracellular and transmembrane domains, T1/ST2 cytoplasmic domain) functions well in the assay upon IL-1 stimulation.

The lack of biological activity shown by the cDNA reported here suggests that it may not be a true ligand for the T1/ST2 receptor and that the interaction between the two proteins may be of no physiological relevance. Many other explanations exist, however. Although we believe that our assays should have responded to a T1/ST2 ligand, it remains possible that the binding protein will display activity in other settings. Alternatively, perhaps the binding protein serves as an antagonist, similar to the IL-1ra, to regulate the activity of an undiscovered primary ligand. Yet another possibility is that the T1/ST2 receptor does not function on its own but requires an accessory molecule, similar to the IL-1R AcP (28), to respond to the binding protein and that this accessory protein is not expressed in 3T3 or COS cells. Finally, the functional interaction was examined in only one direction, *i.e.* the effect of the binding protein on T1/ST2 receptor-bearing cells. Instead, induction of a biological signal may occur in cells bearing the membrane-bound binding protein. At the moment, we are unable to distinguish among these hypotheses.

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